Cell, Vol. 106, 271–274, August 10, 2001, Copyright ©2001 by Cell Press

Dendritic Cells as Vectors for Therapy

Minireview

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Vaccines against infectious diseases are the success story of immunology. They have eradicated smallpox and spared countless people from tetanus, measles, polio, and hepatitis. Consequently, there is a hope for the generation of effective cancer vaccine(s). However, cancer vaccines should be therapeutic in early (including premalignant) and advanced cancer and should induce cellular and not just humoral immunity.

Vaccines are composed of antigen(s) and adjuvant(s). Adjuvants play a critical role in determining the quantity and quality of the immune response to the antigen. Identification of appropriate adjuvants represents a universal problem in vaccine development. For example, aluminum hydroxide (AlOH), currently the standard adjuvant for prophylactic vaccination to infectious diseases induces type 2 T cell (Th2) and antibody responses. However, Th2 immunity and antibody responses may be harmful. Classic examples include Dengue and respiratory syncytial virus (RSV) vaccines that cause higher morbidity upon subsequent exposure to the virus in vaccinated (RSV) or naturally infected (Dengue) patients. Furthermore, AlOH does not induce Th1 and cytotoxic T cell (CTL) responses that are necessary for effective antitumor immunity. Other adjuvants such as QS-21, GM-CSF, and incomplete Freund's adjuvant have permitted, in some instances, specific CD8+ T cell responses when injected with tumor antigens; these responses were, however, detectable only after in vitro restimulation (Bendandi et al., 1999; Rosenberg et al., 1998; Simon et al., 2001). Dendritic cells (DCs), the topic of this review, are "Nature's adjuvants" and, as such, represent an essential component of any vaccination strategy.

DCs were originally discovered as antigen presenting cells critical for the induction of primary T cell-dependent immune responses (Steinman, 1991). Immature DCs in peripheral tissues, the Langerhans cell in the skin being the first example, can capture antigen(s) and can sense "danger" signals (pathogens, tissue damage, and local inflammation), which trigger their maturation. DCs process captured or intracellularly produced antigen(s) into peptides, migrate via afferent lymphatics to lymph nodes, and present MHC-peptide complexes to naive T cells (Banchereau et al., 2000; Steinman, 1991). DCs also control the type of immune response, i.e., cytokines produced by helper or cytotoxic T cells: IFN-γ (type 1), IL-4 (type 2), or IL-10 (regulatory T cells). Such control can be influenced by the subset of DC and the type and duration of the maturation signals they receive. Evidence is starting to accumulate for the role of DCs in the induction of immunological tolerance. A provocative recent example is the observation that immature DCs are not simply ignored by the immune system, but can lead to tolerance by inducing IL-10 producing, regulatory T cells (Dhodapkar et al., 2001; Jonuleit et al., 2000). Therefore, the biology of DCs offer several targets for the control of cellular immunity.

Immunization strategies in cancer and infectious diseases may frequently have as a common denominator the targeting of DCs. However, it will be critical to consider the function of distinct DC subsets (Figure 1), *and* induction of appropriate maturation and migration. If the vaccine goes to the "wrong" DC subset and/or fails to induce its maturation, there might be no protective immunity, and possibly the induction of tolerance. The functions of DC subsets will be considered below. We will emphasize recent studies that exploit DCs generated ex vivo, charged with antigen, and injected back into animals or humans to manipulate immunity. Observations from these studies together with advances in DC biology will teach us how to manipulate DCs in vivo.

Vaccination with DCs to Improve Immunity

Inaba and colleagues demonstrated that the injection of DCs, charged with antigen ex vivo, could sensitize normal mice to protein antigens (Steinman, 1991). This seminal work also suggested that using DCs directly as a vaccine might best circumvent the problem of variable in vivo DC targeting. The immunogenicity of antigens delivered on DCs has now been demonstrated in human studies. Indeed, single s.c. immunization of healthy volunteers with 2–4 $\times$ 10^6 antigen-loaded mature monocyte-derived DCs rapidly expanded CD8+ and CD4+ T cell immunity. A single boost several months later led to expansion of CTL with increased affinity against viral peptide, an observation never made with any other vaccination strategy so far (Dhodapkar et al., 2000).

There is a large body of literature involving animal models of tumor immunity in which DCs loaded with tumor associated antigens (TAA) are able to induce protective antitumor responses. When tested, DCs can be superior to other vaccination strategies (Gilboa, 1999). There also are reports in which DC immunization produces significant therapeutic immunity to established tumors. A number of trials have now utilized TAA-loaded DCs as vaccines in humans. Some clinical and immune responses (T cell proliferation and DTH) without any significant toxicity have been observed in early studies (Hsu et al., 1996; Nestle et al., 1998). More recent DC vaccination studies put further emphasis on the elicited immune responses and have included control antigens for CD4+ and CD8+ T cell responses. The latter help to verify that the DCs are immunogenic, and that the patient's immune system is competent to mount an immune response. The Erlangen group demonstrated that T cell immunity to both control antigens (viral peptide and bacterial protein) and melanoma peptide can be

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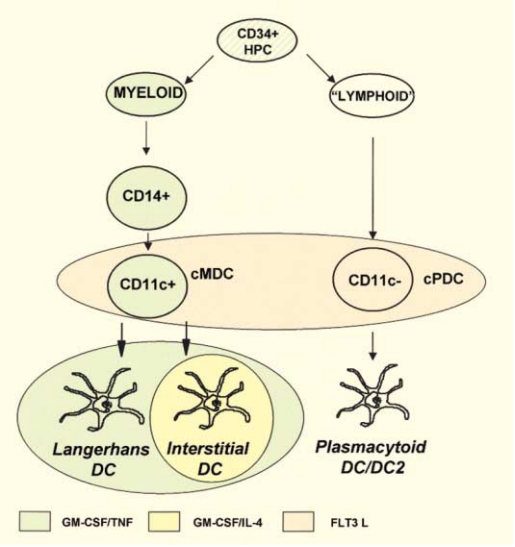

Figure 1. Subsets of Human Dendritic Cells Used in Clinical Trials

Blood DCs, mobilized by FLT3 ligand, contain both CD11c+ myeloid DC and CD11c- plasmacytoid DC. Most studies to date have been carried out with DC made by culturing monocytes with GM-CSF and IL-4. These preparations contain cells that resemble intDCs and are devoid of LCs. These DCs are immature and require exogenous factors (CD40 ligand or macrophage cytokines) for maturation. DCs can also be generated by culturing CD34+ HPC with GM-CSF and TNF-α that permits to obtain two DC subsets: LCs and intDC. Adding IL-4 to CD34 cultures with GM-CSF/TNF skews differentiation toward intDCs.

induced, even in patients with advanced stage IV melanoma, by vaccination with antigen-pulsed, mature monocyte-derived DC (Thurner et al., 1999). Furthermore, when these DCs were loaded with MHC class II binding melanoma peptides, strong tumor-specific Th1 responses were elicited. IFN-γ secreting Th1 cells are likely to be critical for more effective and long-lasting anti-tumor immunity. The Dallas group has demonstrated that CD34+ stem cell-derived DCs, pulsed with control antigens and multiple melanoma peptides, induce primary and recall immune responses detectable directly in the blood in patients with stage IV melanoma. It was observed that the level of immune responses in the blood correlated with early outcome at the tumor sites, thus providing further stimulus for the idea that the measurement of immune responses in the blood helps evaluate vaccine efficacy. Many prior studies have reported some responses in the blood, but often in the minor fraction of the vaccinated cancer patients, or only after prolonged restimulation with antigen in culture. Although the new results with antigen-bearing DCs are encouraging, DC vaccination is at an early stage, and several parameters need to be established (Figure 2).

Parameters of Dendritic Cell Vaccines

The Subsets of DC. The concept of distinct DC subsets in humans (Figure 1) came from analyses of skin DCs, DCs generated in vitro by culture of CD34+ stem cells, and blood DC precursors (Banchereau et al., 2000). Human skin contains epidermal Langerhans cells (LCs), characterized by the expression of CD1a and by Birbeck granules, and interstitial (dermal) DCs, lacking Birbeck

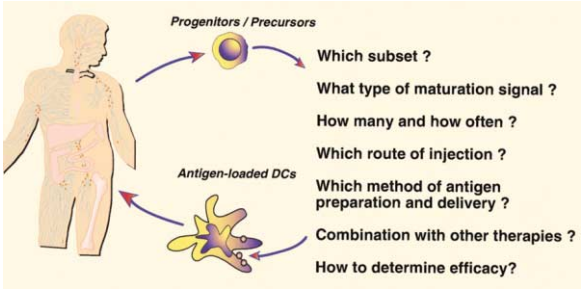

Figure 2. DC-Based Vaccines: Parameters to Monitor

granules but expressing coagulation factor XIIIa. These two subsets also emerge in cultures of stem cells with GM-CSF and TNFα (Caux et al., 1996). While both subsets can induce naïve CD4+ T cell proliferation, only interstitial DCs produce IL-10 and can induce the differentiation of naïve B cells into immunoglobulin-secreting plasma cells. While no unique function has yet been formally attributed to LCs, there are hints they may be particularly efficient activators of cytotoxic CD8+ T cells. The majority of clinical studies to date have been carried out with ex vivo generated monocyte-derived DC (Banchereau et al., 2000), which resemble a single subset, i.e., interstitial DCs. Another example of DC subsets is evident in fresh human blood, each representing a small fraction (~0.3%) of the mononuclear cells. One terminology for these subsets is CD11c+ myeloid DCs, and CD11c- plasmacytoid DCs. The CD11c+ cells are thought to be similar to monocyte derived DCs, while the CD11c- plasmacytoid cells are distinct in their capacity to make very large amounts of IFN α, but much lower amounts of IL-12. IFN-α and IL-12 are two cytokines that are part of the innate response to many pathogens, and they in turn influence many other aspects of cell-mediated immunity. The efficacy of these distinct human DC subsets will need to be compared in clinical studies.

The Optimal DC Maturation State and Stimulus. Immature DCs are weak immunogens. Indeed, the Erlangen group has observed that intranodal injection of immature DCs does not lead to significant immune responses, contrary to the intranodal injection of mature DCs in the same patient. Immature DCs can be tolerogenic. Indeed, injection of immature DCs in healthy volunteers leads to the inhibition of CD8+ T cell immunity to viral peptide with the appearance of peptide-specific IL-10 producing T cells (Dhodapkar et al., 2001). In contrast, mature DCs (triggered for instance by a mix of macrophage products such as IL-1β/IL-6/TNFα/PGE2) induce functionally superior CD8+ T cells and polarize CD4+ T cells toward IFN-γ production. Thus, DC maturation is a critical parameter for the use of these cells in active immunization of patients. It will be important, therefore, to identify stimuli that trigger an equally effective maturation program in the various human DC subsets.

DCs Dose, Frequency, and Route of Injections. In human trials published so far, the DCs were usually given at 2–4 week intervals, and at doses between 4–40 million without striking differences in results. In vitro studies on human T cell activation by DCs would predict that

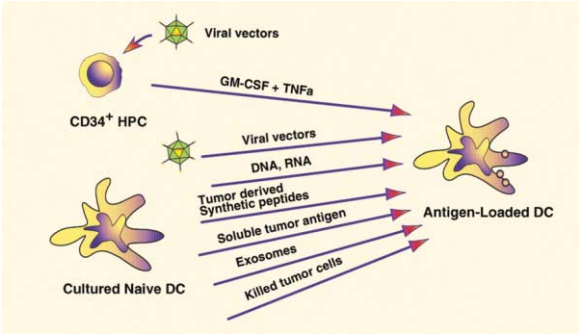

Figure 3. Loading Antigens on Dendritic Cells

higher doses of DCs given more frequently should provide more intense and durable TCR triggering and thus promote T cell priming and polarization. However, frequent stimulation might cause activation-induced death of T cells. Also, the induced CTL may kill booster DCs and thus reduce efficacy. Yet, fully mature DCs seem resistant to CTL lysis.

Other important factors are the route of injection and the migration of DCs from the injection site. While antigen-loaded DCs may prime T cell responses regardless of the route of injection, the quality of responses may be affected by it, with predominant Th1 responses after i.d. and intralymphatic administration, but unpolarized T cell and antibody responses upon i.v. administration (Fong et al., 2001). In this way, one can investigate the requirements, for example, of improving migration at the level of specific chemokines like CCL19 and CCL21 that interact with CCR7 on maturing DCs.

A question that has yet to be addressed is the duration of DC-based vaccination against cancer. How long shall we keep immunizing patients in whom vaccine induces immune responses and disease stabilization? Preliminary results from the Dallas group show that some of the patients who had a partial response to DC therapy after the initial 4 injections experienced further tumor regression after 4 additional vaccines. Possibly, consolidation vaccine therapy should be lifelong, and may require adjunctive immune therapies such as cytokines to support T cell memory.

Source, Preparation, and Antigen Loading Strategy. Several systems have been employed to load DCs with TAA (Gilboa, 1999) (Figure 3). Loading MHC class I molecules with peptides derived from defined antigens is most commonly used, and is also applied to recently identified MHC class II helper epitopes. Although important for "proof of concept" studies, the use of peptides has limitations coming from (1) their restriction to a given HLA type, (2) the limited number of defined TAA, and (3) the induction of a restricted repertoire of T cell clones less able to control tumor antigen variation. Furthermore, quantity and longevity of peptide loading is difficult to control; the use of antibodies to MHC class I/peptide complexes may help in this aspect. This could also help determine the relationship between MHC-peptide density and magnitude/affinity of induced T cell responses, and to standardize vaccines in large multicenter trials. Alternative strategies that provide both MHC class I and class II epitopes and lead to a diverse immune response involving many clones of CD4+ T cells and CTL are needed. These include: recombinant proteins, exosomes (vesicles rich in MHC/peptide complexes and heat shock proteins), viral vectors, plasmid DNA, or RNA transfection (Fong and Engelman, 2000; Gilboa, 1999). Yet another way is to exploit the capacity of DC to present peptides from phagocytosed dead tumor cells on both MHC class I and II molecules (Larsson et al., 2001). Tumor death may unravel subdominant or cryptic epitopes which, when processed and presented by DC, may either reach the proper activation threshold for memory T cells or lead to priming of naïve T cells. We would predict that in advanced metastatic disease, using tumor cells as antigen source will be particularly helpful, while defined antigens would be better for the adjuvant/minimal residual disease setting, following, for instance, removal of a high-risk primary melanoma or resection of lymph node metastasis.

Vaccine Efficacy and the Quality of Immune Response. A difficult aspect of immunotherapy protocols is the identification of immunologic markers that will permit prediction of clinical efficacy. While the ultimate efficacy of cancer vaccine should be measured by the duration of disease free survival, or at least the time to disease progression, these end points require sufficiently long follow-up. Hence, the necessity for "surrogate markers" that would be predictive of clinical outcome. Progress with vaccines against infectious diseases depended on measuring antibody responses as "surrogate markers"; hence, progress with vaccines against cancer may be advanced by detailed measurement of elicited T cell responses in the blood.

The quality of the induced immunity is important. The measurement of antigen-specific CD8+ T cells, using MHC-tetramers, helps to quantify antigen-specific responses but additional data are needed to assess functional activity. Cytotoxic T cell function is often evaluated on peptide-pulsed target cells, but it will be valuable to assess recognition of endogenously expressed antigens using tumor cell lines and, if possible, primary tumor cells. More studies are needed to compare response in the blood with that in the tumor. Skewing of T cell phenotype needs to be considered as well. For example, the differential expression of CCR7 and CD45 isoforms may help characterize the functional status of antigen-specific T cells, i.e., CCR7+ T cells (central memory) will most likely migrate to lymph nodes while the shift toward CCR7- phenotype (effector memory) should be associated with migration to the tissue and possibly faster differentiation into CTL capable of killing tumor cells. Other immune effectors also need to be taken into account including CD4+ T cells, NKT and NK cells, as well as B cells. For instance, IFN-γ producing CD4+ T cells can inhibit tumor-induced angiogenesis. Induction of NKT cells that kill a wide spectrum of tumor cells or NK cells that recognize MHC class I deficient tumor cells could be desirable, yet caution must be taken with regard to the cytokines that they produce. Indeed, IL-13-producing NKT cells may inhibit CTL-mediated tumor elimination and favor tumor progression (Terabe et al., 2000). Finally, B cells may inhibit the induction of T cell-mediated tumor immunity by competing with DCs for uptake of tumor-derived antigens

(Qin et al., 1998). Yet, in the active immunization setting, there may be desirable humoral responses and we certainly need to learn more about the types of humoral immunity induced by the different DC subsets.

There are concerns that repeated immunization with DCs carries the risk of developing autoimmunity, particularly when targeting shared tumor antigens. DC-based active immunization will clarify this issue, including the potential value of eliciting a strong response of limited duration to an antigen shared by the tumor and self tissue.

In Vivo Manipulation of Dendritic Cells: The Targeted Vaccines

Vaccination with ex vivo generated DCs is not feasible for large-scale immunization, either in cancer or in infectious diseases, e.g., malaria. Thus, there is a need to develop strategies that can provide a robust protective/therapeutic immune response of optimal type with minimal amounts of vaccine and limited boosting. Research in this area may benefit from the ability to (1) mobilize large numbers of DCs in vivo (DC-poietins) and (2) deliver mobilized DCs to antigens and activation molecules ("intelligent missile").

DC-poietins are the cytokines that mobilize DCs in vivo: they either increase their numbers (G-CSF, GM-CSF, and FLT3 ligand) or activate them (IFN-α) (Pulendran et al., 2001). These cytokines also mobilize other immune effectors, for instance neutrophils (G-CSF, GM-CSF), macrophages (GM-CSF), and NK cells (Flt3-L). Therefore, they can be expected to boost both the non-antigen-specific innate immunity and the antigen-specific adaptive immunity. Indeed, administration of FLT3 ligand to mice enhances vaccine immunogenicity (in an HIV model) as well as protects the mice against viral rechallenge (in a Herpes virus model). IFN-α has long been recognized for its immunomodulatory activity, though its adjuvancy was only recently assigned to in vivo DC activation (Le Bon et al., 2001).

DCs may be targeted in vivo by "intelligent missile," a generic vaccine equipped with (1) the immunogens, the optimal antigenic preparations that can be targeted to desired MHC molecules; (2) DC activation molecules and specific ligands that would permit targeting of the desired DC subset. One might take advantage of molecules that bind to pattern recognition receptors, like the Toll-like receptors (TLRs), through which the immune system senses microbial products and/or tissue damage. For example, CpG oligonucleotides can activate DCs in vivo while heat shock proteins can both activate DCs and chaperone the peptide. Furthermore, as the TLRs are differentially expressed on DC subsets, their ligands could serve as targeting molecules; and (3) molecules determining the quality and the type of the immune response, for instance, chemokines attracting naïve T cells, costimulatory molecules, as well as type 1/type 2 skewing molecules.

Conclusions

DCs are an attractive target for therapeutic manipulation of the immune system, to enhance insufficient immune responses, in infectious diseases and cancer, or attenuate excessive immune responses, in allergy and autoimmunity. However, the complexity of the DC system brings about the necessity for its rational manipulation to achieve protective or therapeutic immunity. Immunization with ex vivo generated DC has proven feasible, and permits the enhancement as well as the dampening of antigen-specific immune responses in man. These ex vivo strategies should help identify the parameters for DC targeting in vivo. Today's studies are also considering the relationship of DCs to the correction of pathologic or undesired immune responses, like allergic and autoimmune diseases, graft rejection, and graft versus host disease.

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